Supplemental Figures and Legends

Figure S1. Sequence alignment of Atp13a2 Isoforms. Accession Numbers: Isoform 1 NP_071372.1, Isoform 2 NP_001135445.1, Isoform 3 NP_001135446.1

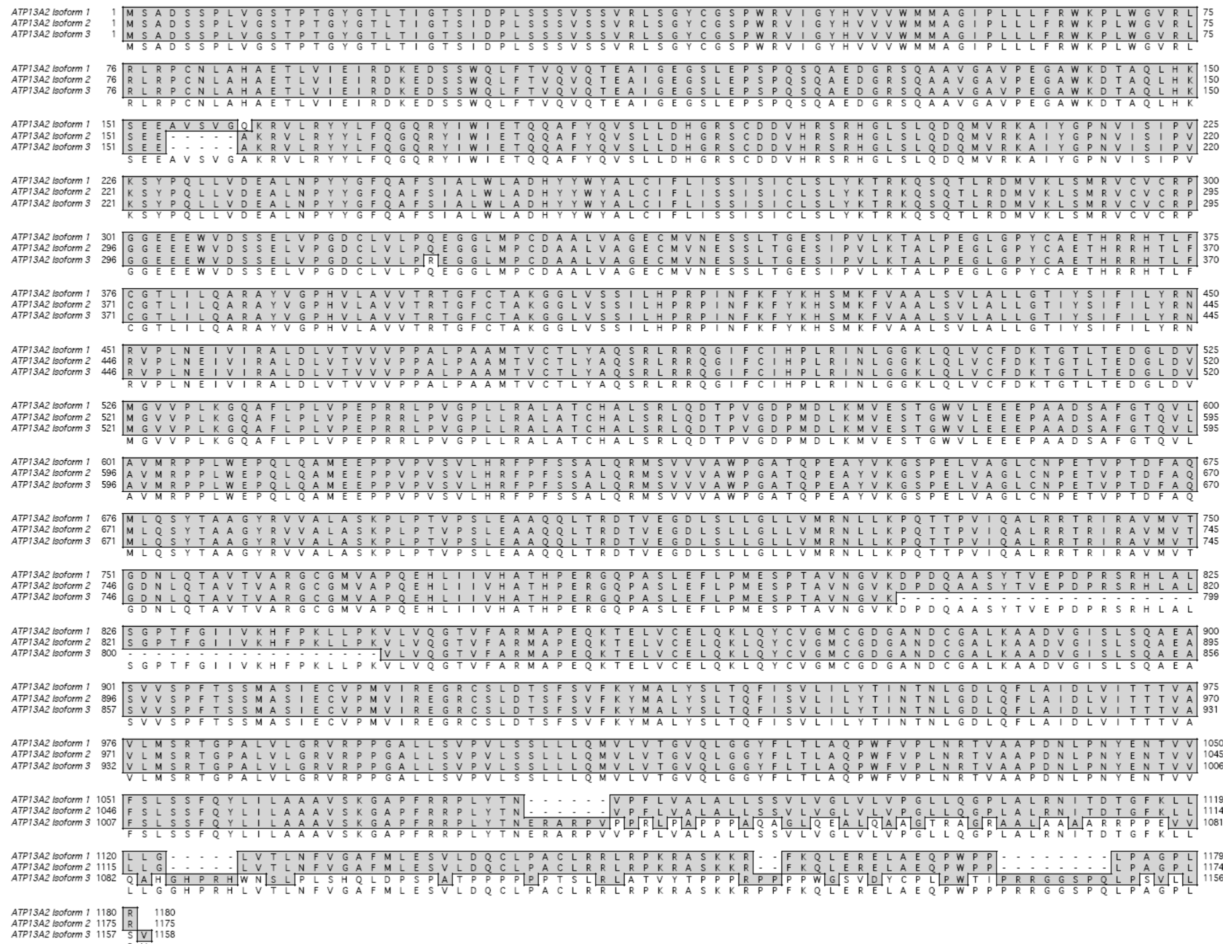


Figure S2. Atp13a2 Hydrophobicity Profiles. (A) Isoform-1. (B) Isoform-2. (C) Isoform-3. The C-terminal region of each isoform is outlined in red. Note Isoform-3 is missing the last two transmembrane domains (eight total) compared to isoforms 1 and 2 (ten total).

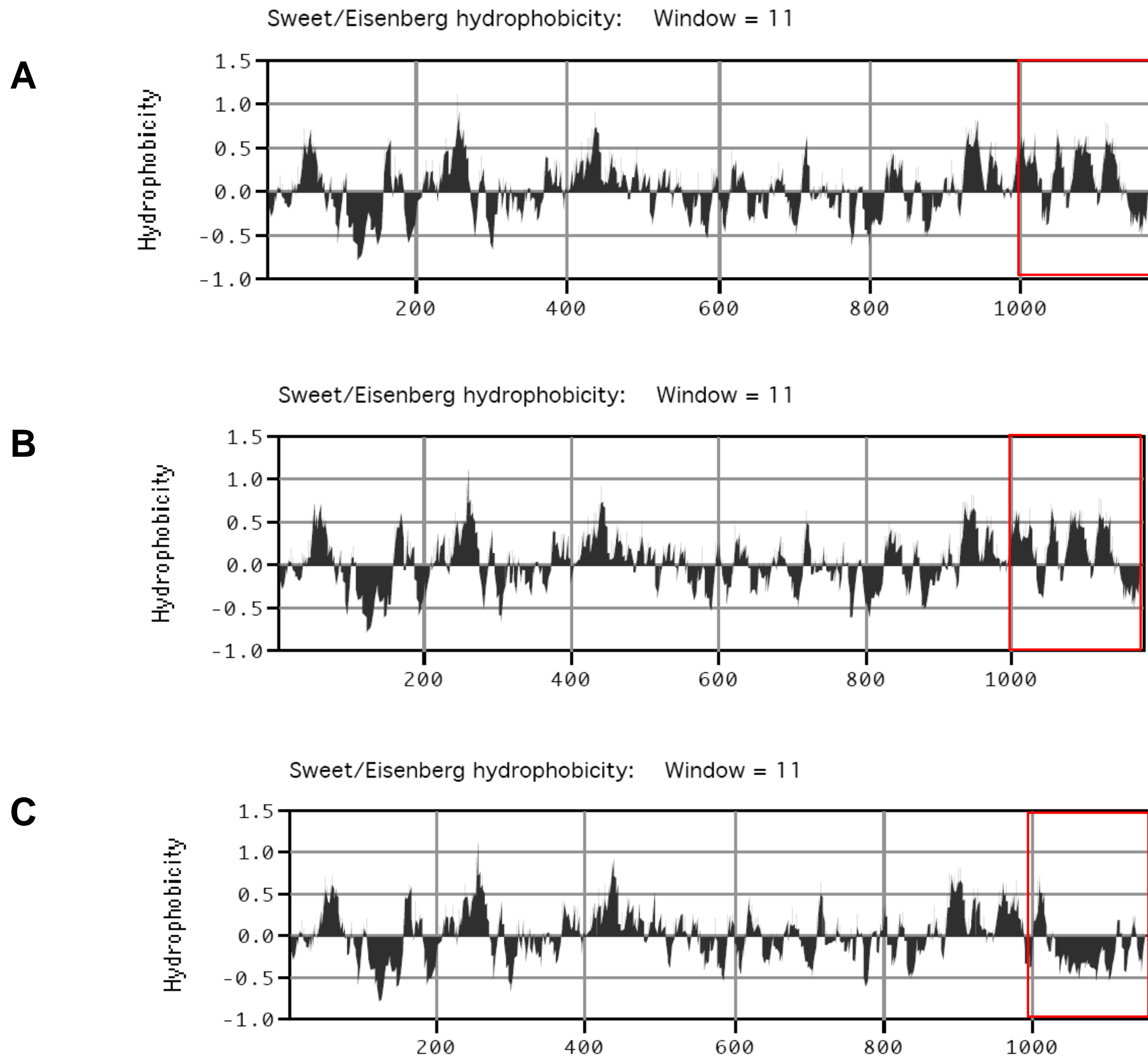


Figure S3. Predicted Atp13a2$^{Isoform\ 1}$ Topology and Mutation Sites**.** Diagram depicts conserved P-type ATPase motifs (colored ovals) and mutation events (colored stars). The conserved domains are as follows: actuator domain (A-domain), the phosphorylation domain (P-domain) and the nucleotide binding domain (N-domain). The P-domain contains the conserved aspartic acid residue which is phosphorylated during the catalytic cycle. The mutation events are as follows: Ex13, an in-frame deletion of exon13 which removes 111 nucleotides and a portion of the third transmembrane domain; Dup22, a duplication of 22 base pairs in exon16 which creates a frameshift and deletes the last 6 transmembrane domains; ΔC, a deletion of cytosine 3057 which results in a frameshift and the removal of the last three transmembrane domains.

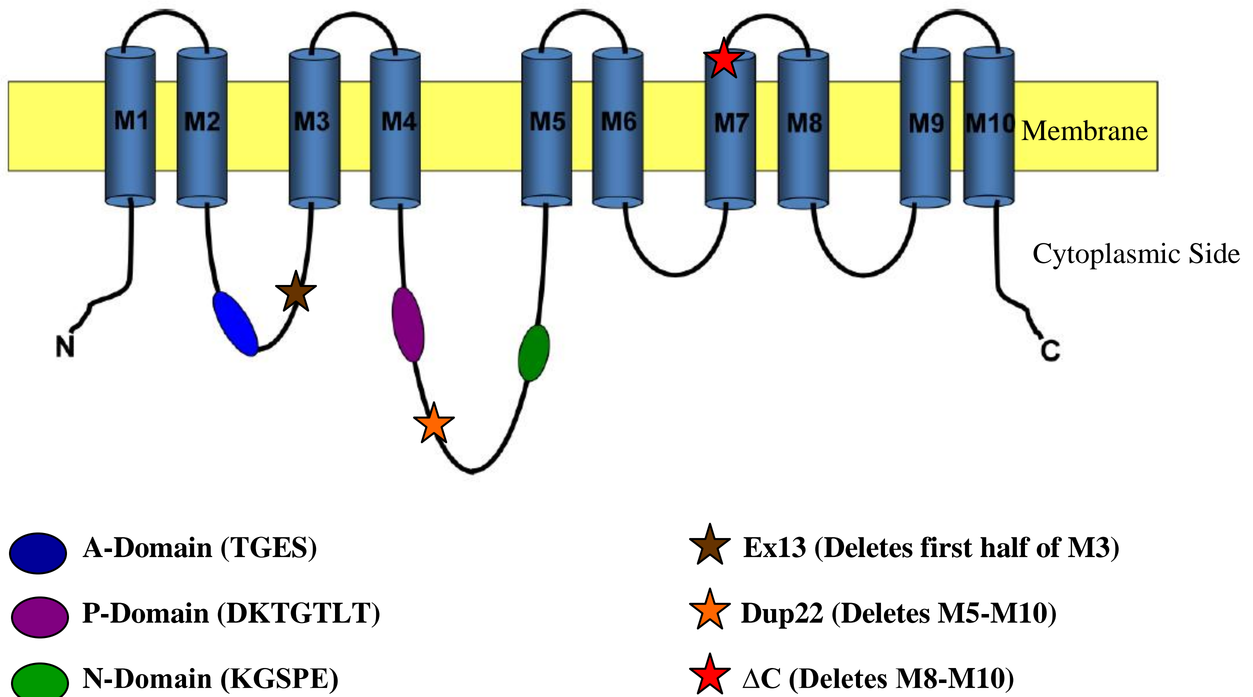
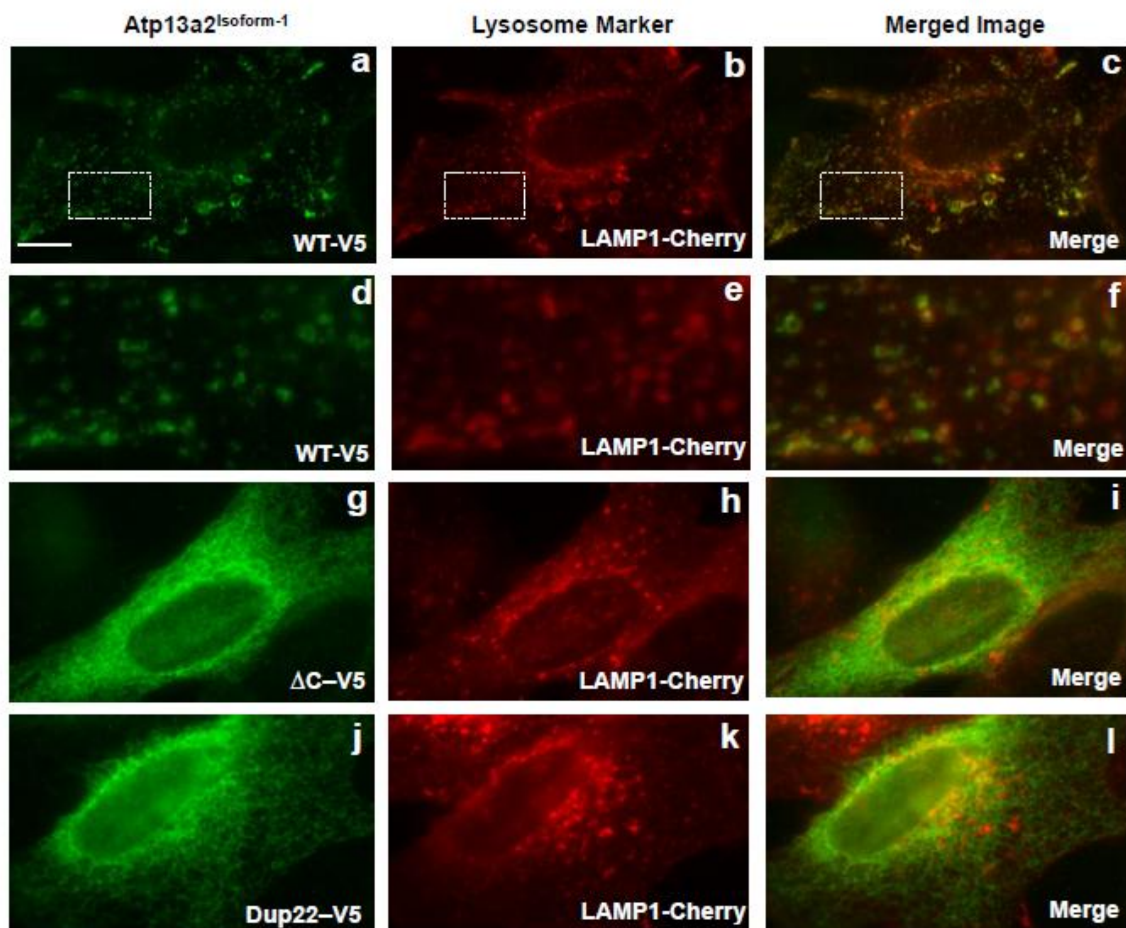


Figure S4. Atp13a2 Localization**.** (A) HeLa cells were cotransfected with LAMP1-Cherry and V5-tagged wild type (a-f), ΔC (g-i) or Dup22 (j-l) Atp13a2$^{Isoform-1}$ and visualized using fluorescence microscopy. (d-f) Magnification of region outlined in panels (a-c). (B) HeLa cells were cotransfected with LAMP1-Cherry and GFP-tagged ΔC (a-c) or Dup22 (d-f) Atp13a2$^{Isoform-1}$ and visualized using fluorescence microscopy. (C) HeLa cells were cotransfected with LAMP1-Cherry and GFP-tagged wild type (a-c), ΔC (d-f) or Ex13 (g-i) Atp13a2$^{Isoform-3}$ and visualized using fluorescence microscopy. Bar, 5μm

A



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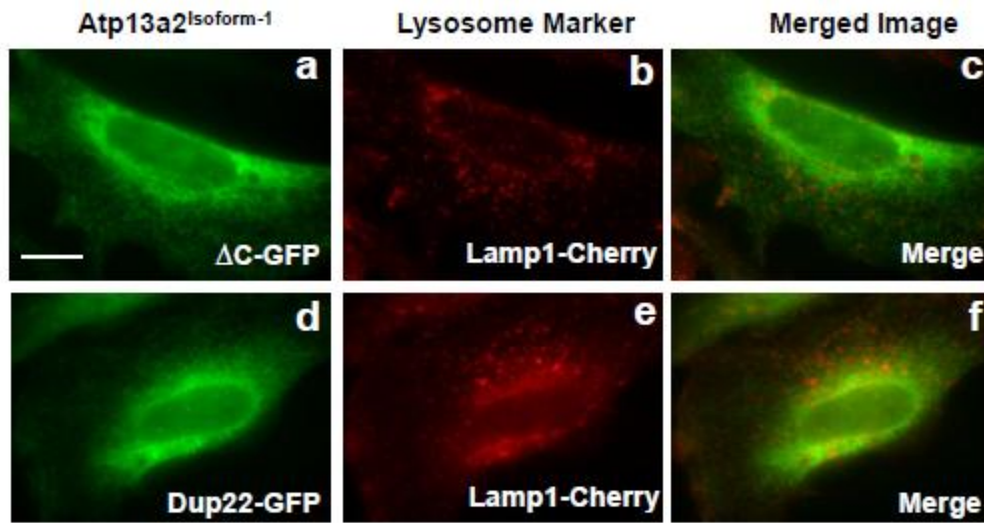

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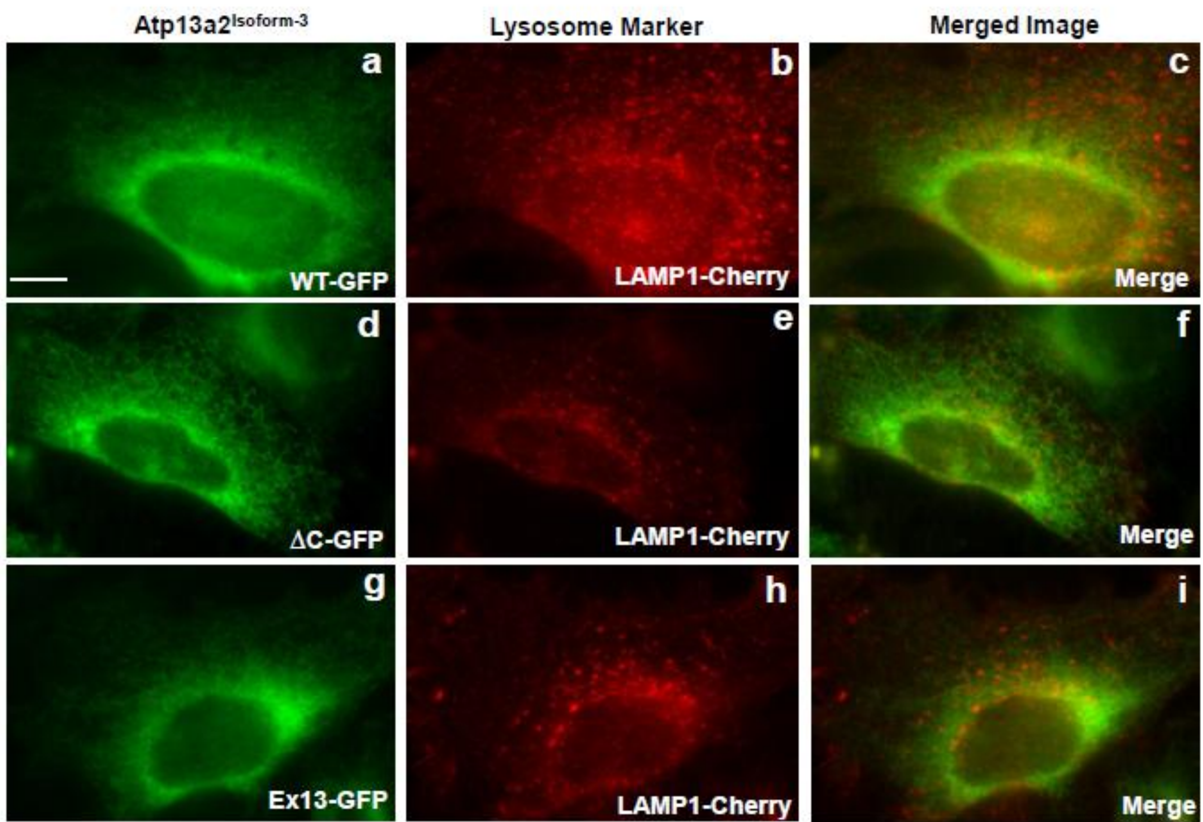

Figure S5. Atp13a2$^{Isoform-1}$ Glycosylation**.** HeLa cells stably expressing V5-tagged wild type ATP13A2$^{Isoform-1}$ were treated with tunicamycin for the indicated time periods and analyzed by immunoblotting.

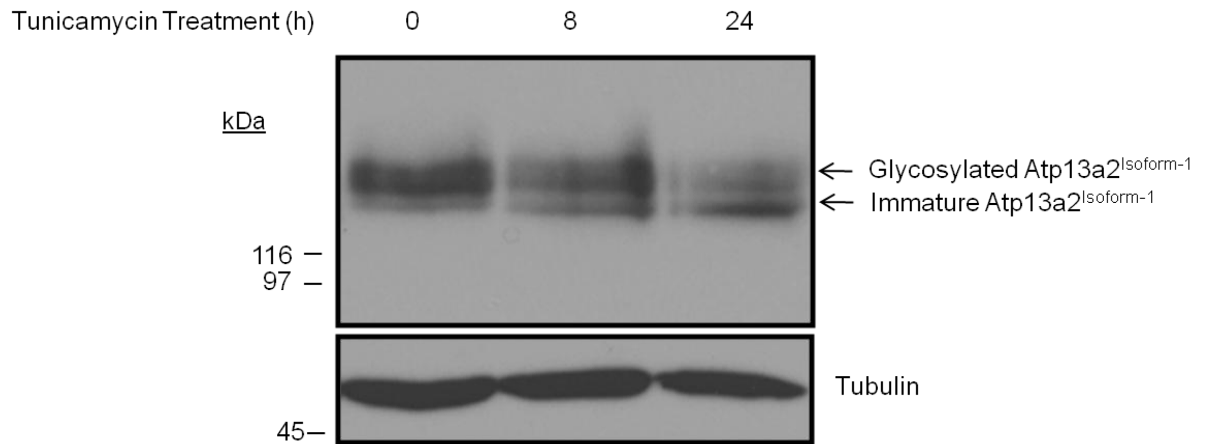

Figure S6. Pulse-chase analysis of Atp13a2$^{Isoform-1}$. (A) HeLa cells were transfected with V5-tagged ΔC Atp13a2$^{Isoform-1}$. Twenty hours after transfection cells were pulse-labeled with [^{35}S]methionine and chased with nonradioactive medium containing MG132 or DMSO (vehicle control) for 0-6 hours. The ΔC protein was immunoprecipitated from the lysates using an anti-Atp13a2 antibody and separated by SDS-PAGE. Radioactivity of the ΔC-V5 band was measured by phosphoimage analysis. (B) Graph representing the radioactivity measured in (A). The estimated half-life of the ΔC protein increased from approximately 1 hour (Control) to 3.5 hours (MG132) which is similar to the results of our CHX-chase experiments. (C) HeLa cells stably expressing V5-tagged wild type Atp13a2$^{Isoform-1}$ were subjected to the same conditions as described above for the ΔC mutant. Phosphoimage analysis of the immunoprecipitates showed the wild type protein to be relatively stable. Attempts to quantify the wild type band were unsuccessful due to the high background present.

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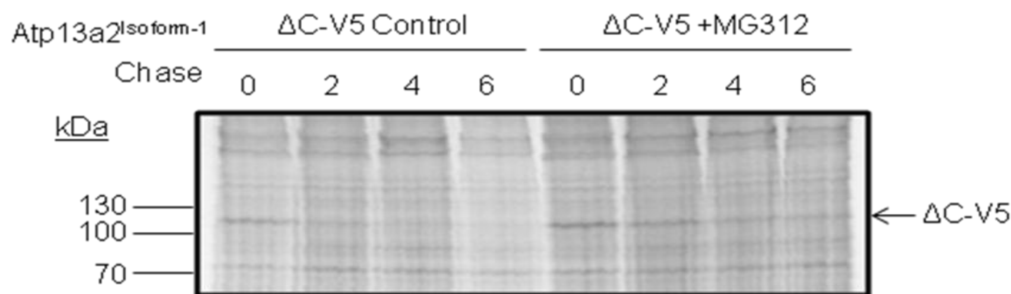

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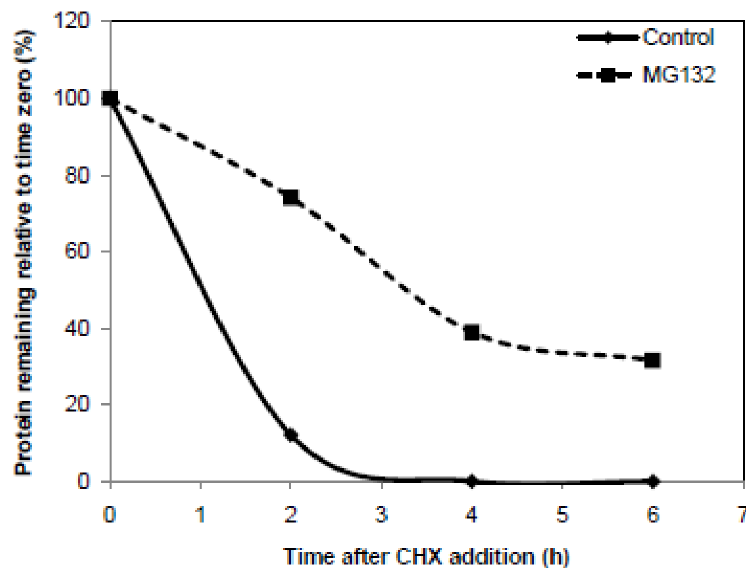

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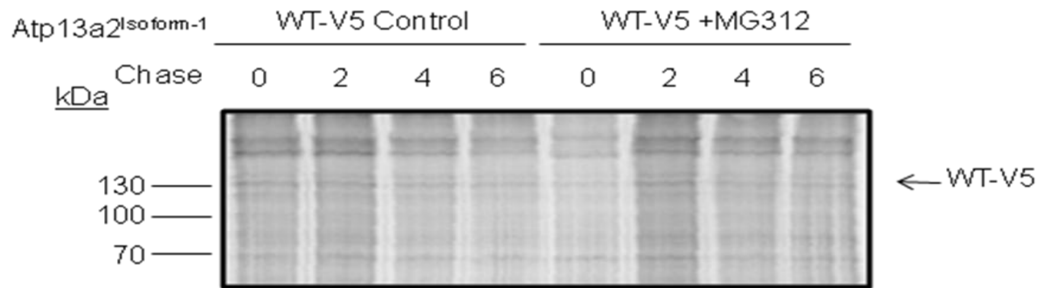

Figure S7. Wild type Atp13a2$^{Isoform-1}$ Turnover. HeLa cells stably expressing V5-tagged wild type Atp13a2$^{Isoform-1}$ were treated with cycloheximide and MG132 or DMSO vehicle for 12 and 24 hours. Lysates were immunoblotted for V5 and tubulin. The half-life of the protein was calculated to be in excess of twenty-four hours.

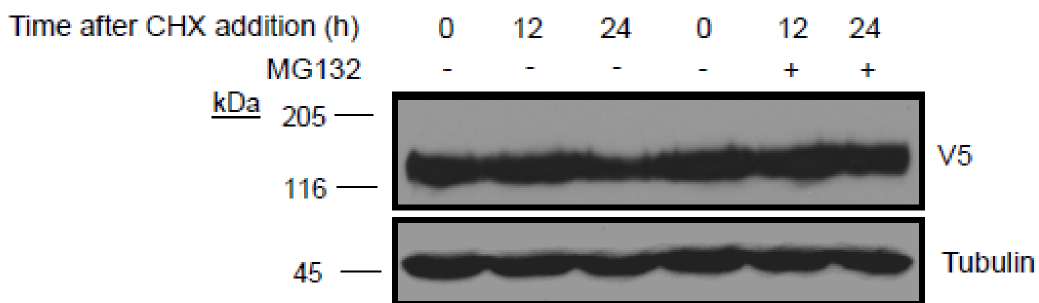

Figure S8. Expression of Atp13a2$^{Isoform-3}$ and Quantification of Cell Death. HeLa cells were transfected with the indicated GFP constructs and cell death assays were performed as described previously for Atp13a2$^{Isoform-1}$. Graph is an average of three independent experiments and the data is shown as the mean ± standard deviation of the mean. The extent of cell death for the different Atp13a2$^{Isoform-3}$ constructs was compared to that of the GFP control. Independent analysis was performed for the tunicamycin treatment and non-treatment. Expression of wild type or mutant Atp13a2$^{Isoform-3}$ results in an significant increase in cell death compared to control GFP cells with or without tunicamycin *$P < 0.0005$ ** $P < 0.005$. By this analysis wild type Atp13a2$^{Isoform-3}$ differs from Atp13a2$^{Isoform-1}$ in that it increases cell death to the same extent as the Ex13 and ΔC mutants.

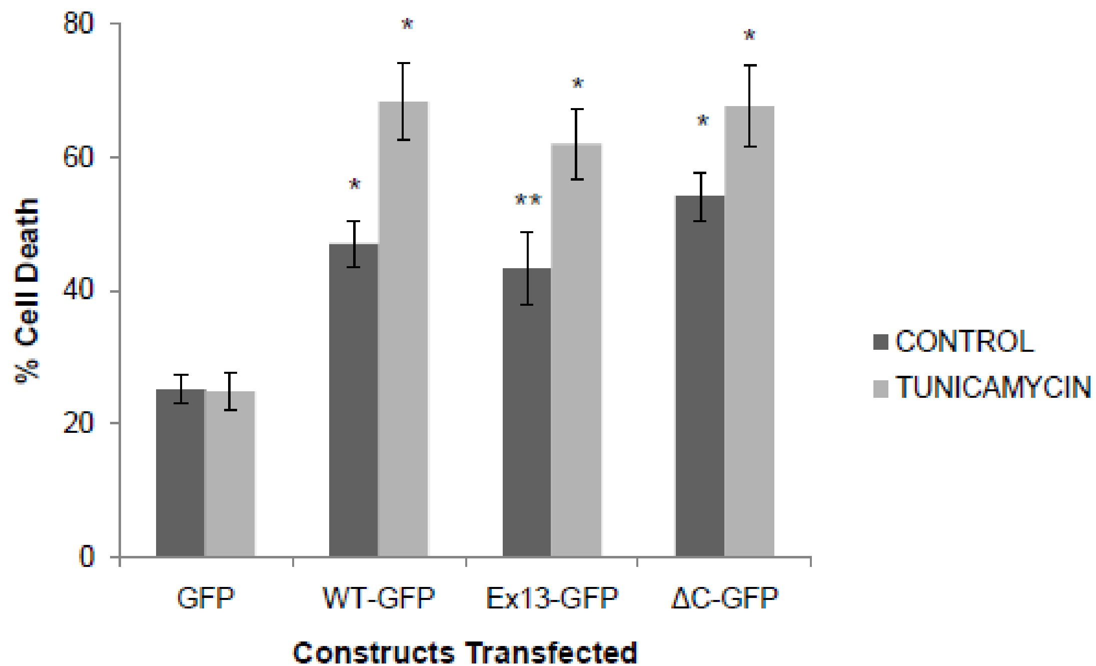

Figure S9. Expression of Atp13a2$^{Isoform-1}$ and Quantification of BiP Levels. HeLa cells were transfected with GFP alone or with the indicated GFP-tagged Atp13a2$^{Isoform-1}$ constructs. Twenty hours after transfection, the cells were treated with tunicamycin or DMSO vehicle control for 8 hours at a final concentration of 2 μg/ml. (A) Equal amount of lysates were separated by SDS-PAGE and immunoblotted for BiP, GFP, and Actin. (B) Quantification of BiP levels in cells that were either treated or not treated with tunicamycin all normalized to the cells transfected with GFP alone without tunicamycin treatment (average of three independent experiments). BiP levels in the cells transfected with the wild type and Atp13a2 mutants were found to increase relative to the cells transfected with GFP alone without tunicamycin treatment. Similar increases were observed in the tunicamycin-treated cells, except that the wild type did not show a significant increase compared to the mutants. However, we should note that the efficiency of transfection differed between the constructs, which could have underestimated the real differences. Data are shown as mean ± standard deviation of the mean. ($^*P < 0.05$).

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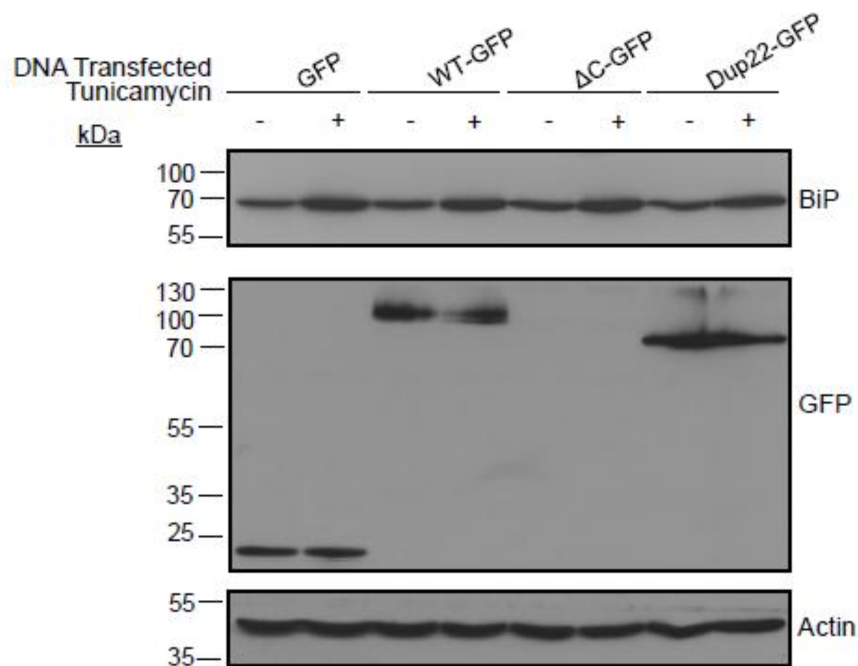

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